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Request for grant of a patent

-5 APR 2002

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08APR02 E709101-3 D02825
P01/7700 0.00-0207975.4

1.	Your reference	300267.GB/JND
2.	Patent application number *(The Patent Office will fill in this part)*	0207975.4
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	GenoVision AS Frysjaveien 40 0884 Oslo Norway
	Patents ADP number *(if you know it)*	818129001
	If the applicant is a corporate body, give the country/state of its incorporation	
4.	Title of the invention	Isolating Nucleic Acid
5.	Name of your agent *(if you have one)*	PAGE WHITE & FARRER
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	54 Doughty Street, London, W C1N 2LS, United Kingdom.
	Patents ADP number *(if you know it)*	1255003

		Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

		Number of earlier application	Date of filing *(day / month / year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body* *See note (d))*	Yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	0
Description	9
Claim(s)	3
Abstract	0
Drawing(s)	1

10. If you are also filing any of the following, state how many against each item.

Priority documents	Not required
Translations of priority documents	Not required
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	Yes
Request for preliminary examination and search (Patents Form 9/77)	No
Request for substantive examination (Patents Form 10/77)	No
Any other documents *(please specify)*	0

11. I/We request the grant of a patent on the basis of this application.

Signature

PAGE WHITE & FARRER

Date 5 April 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr J N Daniels
(020) 7831-7929

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ISOLATING NUCLEIC ACID

The present invention relates to a process for isolating nucleic acid from a nucleic acid-containing sample, and to a kit therefor.

Procedures involving nucleic acids such as DNA and RNA continue to play a crucial role in biotechnology. Nucleic acid detection and manipulation including hybridisation, amplification, sequencing and other processes generally require nucleic acid to have been isolated from contaminating material. Where a nucleic acid-containing sample is a biological sample, contaminating material may include proteins, carbohydrates, lipids and polyphenols. Accordingly, a variety of approaches have hitherto been used in the isolation of DNA or RNA.

Early methods of isolating nucleic acid involved a series of extractions with organic solvents, involving ethanol precipitation and dialysis of the nucleic acids. These early methods are relatively laborious and time-consuming and may result in low yield. Isopropanol may also be used in the precipitation of the nucleic acid.

US5234809 describes a procedure to isolate DNA from biological samples which uses a chaotropic agent together with a silica based nucleic acid binding solid phase. Guanidine hydrochloride at pH 3 to 5 or guanidine thiocyanate at higher pH, combined with other salts, is used as the chaotropic agent. After binding of the DNA to the solid surface, the solid phase may be washed with the

chaotropic agent to remove any biological contamination followed by treatment with 70% ethanol to remove the chaotrope. The DNA is eluted using water.

A variant on this methodology is described in US6027945. Here, a method is described which also uses a silica-based nucleic acid binding solid phase in the presence of a chaotrope to isolate nucleic acid. According to this method, the silica-based solid phase is magnetic, thereby facilitating separation of the solid phase containing the target nucleic acid from the liquid phase containing contaminants upon application of a magnetic field.

WO96/18731 also uses magnetic particles to bind nucleic acid. In this disclosure the magnetic particles are polystyrene-based and polyurethane-coated and a detergent is used instead of a chaotrope.

In spite of the advances made using nucleic acid binding solid phases, the yield of target material can sometimes be undesirably low. The present invention addresses this disadvantage of the prior art.

Accordingly, in a first aspect, the present invention provides a process for isolating nucleic acid from a nucleic acid-containing sample, which comprises:

(a) providing a chaotrope;

(b) providing a nucleic acid binding solid phase capable of binding nucleic acid in the presence of the chaotrope;

(c) providing a source of NH_4^+ or NH_3;

(d) contacting the sample with the nucleic acid binding solid phase in the presence of a liquid phase comprising the chaotrope and the NH_4^+ or NH_3; and

(e) optionally separating the solid phase with the nucleic acid bound thereto from the liquid phase.

In a second aspect, the present invention provides a kit for isolating nucleic acid from a nucleic acid-containing sample, which kit comprises:

(a) a chaotrope;

(b) a nucleic acid binding solid phase capable of binding nucleic acid in the presence of the chaotrope; and

(c) a source of NH_4^+ or NH_3.

It has surprisingly been found that the presence of NH_4^+ or NH_3 in the process for isolating nucleic acid gives an increased yield of nucleic acid compared to cases where NH_4^+ or NH_3 are absent.

Without wishing to be bound by theory, it is thought that the addition of ammonia or ammonium to, say, the chaotropic binding solution, causes the pH to increase by one unit (*i.e.* from 7.5 to 8.5). However, the resulting increased yield of isolated nucleic acid is not believed to be purely a pH effect. If the pH of the chaotropic solution is increased to 8.5 simply by the addition of alkali, this does not affect the yield of isolated nucleic acid. However, the pH of the solution in the presence of ammonia or ammonium does have an effect on the increased yield of the isolated nucleic acid. Adjusting, say, the chaotropic solution containing ammonia or ammonium back to pH 7.5 with

acid does tend to reduce the yield of isolated nucleic acid. Moreover, if the pH exceeds 9.5, the yield of isolated nucleic acid tends to drop. Accordingly, it is preferred that the step of contacting the sample with the nucleic acid binding solid phase in the presence of NH_4^+ or NH_3 is conducted at a pH in the range 8.5 to 9.5.

Instead of NH_4^+ or NH_3, an amine may be used, preferably a primary amine.

The nucleic acid-containing sample typically comprises a biological sample such as a cellular sample. The biological sample may or may not need to be pretreated, depending on its structure. For example, in the case of plant or fungal cells or solid animal tissue, pretreatment would be required as is known in the art. Samples stored in the form of a solid phase such as a paraffin section may also need pretreatment. Samples may be from foodstuffs, environmental samples or clinical samples and may contain prokaryotic or eukaryotic cells or other moieties such as mycoplasmas, protoplasts or viruses. Blood products are an important area for nucleic acid isolation and the present invention is particularly applicable to whole blood and other blood products such as plasma, serum and buffycoat.

The nucleic acid to be isolated may be DNA, RNA or a modified form thereof. Where the nucleic acid is DNA, this may be ds or ss DNA. Where the nucleic acid is RNA, this may be rRNA, mRNA or total RNA.

The chaotrope generally comprises a chaotropic ion provided at a concentration sufficiently high to cause the nucleic acid to lose its secondary structure and, in the case of double-stranded nucleic acids, to melt. Chaotropes are thought to disrupt hydrogen-bonding in water so as to make denatured nucleic acid more stable than its undenatured counterpart. The chaotrope typically comprises a guanidinium salt, urea, or an iodide, chlorate, perchlorate or (iso)thiocyanate. Preferred chaotropes include guanidinium thiocyanate, and guanidinium hydrochloride.

The concentration of chaotrope typically present when contacted with the sample is in the range 2M to 8M.

The nucleic acid binding solid phase must be capable of binding nucleic acid in the presence of the chaotrope but is not limited to any specific material. Various materials are now known as nucleic acid binding solid phases and these include silica-based materials such as those described in US5234809, polymeric materials including latex and polystyrene-based materials such as those described in WO96/18731 and other materials such as glasses.

The form of the solid phase includes sheets, sieves, sinters, webs and fibres. Particles are particularly useful as these may be packed in a column or used in suspension and have high binding capacity. Magnetic particles are particularly preferred because of the ease with which they merely separated from an associated liquid phase in a magnetic field. Typical materials for use in magnetic particles include magnetic metal oxides especially

the iron oxides. Useful magnetic oxides include iron oxides in which, optionally all or a part of the ferrous iron thereof is substituted with a divalent transition metal such as cadmium, chromium, cobalt, copper, magnesium, manganese, nickel, vanadium and/or zinc. Silica-based magnetic particles useful in the present invention include those described in US6027945 and US5945525.

The source of NH_4^+ or NH_3 is typically an ammonia solution although other possible sources include those capable of generating ammonia by a chemical reaction or transformation. In order for the NH_4^+ or NH_3 to be present when the sample is contacted with the nucleic acid binding solid phase, there is no particular limitation on how the NH_4^+ or NH_3 should be provided. Conveniently, the NH_4^+ or NH_3 can be provided with the chaotrope, although the technical effect provided by the invention also allows the NH_4^+ or NH_3 to be provided with the solid phase or even the sample. A potential advantage does arise if the chaotrope and NH_4^+ or NH_3 are provided together, however. The process according to the invention may further comprise a lysis step comprising subjecting the biological sample to conditions to lyse the sample. This is typically carried out so as to disrupt cells and release their nucleic acid. Lysis conditions conveniently involve the presence of a detergent. It is thought potentially advantageous for the NH_4^+ or NH_3 to be present during the lysis step as this may have the beneficial effect of increasing yield of nucleic acid during this step. It is also convenient to have the chaotrope present at the same time as this can help the lysis step. Accordingly, where the chaotrope and the NH_4^+

or NH_3 are provided together as a solution, this solution can be used to treat the biological sample during the lysis step.

The step of separating the solid phase with the nucleic acid bound thereto from the liquid phase is generally required in order to remove contaminants in the liquid phase. Further washing steps may be applied to the solid phase at this point. Any conventional separation step for separating solid phase from liquid phase is applicable, including centrifugation and decanting of the liquid phase from the pelleted solid phase or using a column in which the solid phase is packed and the liquid phase passed through. Where the magnetic solid phase is used, this facilitates separation, which can be carried out in the presence of a magnetic field.

Depending on the form in which the isolated nucleic acid is required, a further elution step can be provided. In some cases it may be satisfactory for the nucleic acid to remain bound to the solid phase. This may be the case if further manipulations of the nucleic acid on a solid phase are required, such as an amplification step. Equally, the nucleic acid may be eluted from the solid phase by applying an elution solution, which may simply be water or a buffer.

The present invention is now described in more detail, by way of example only, with reference to the following Example and accompanying figure.

Example

The magnetic particles. Magnetic Silica particles were obtained in accordance with UK patent application no. 0116359.1 filed on 4th July 2001.

The chaotropic lysis and binding solution. To 130 g Guanidine thiocyanate (Sigma) was added 95 ml 0.1 M TRIS HCl pH 7 (Sigma) + 8 ml 0.5 M EDTA (Invitrogen) and 2.5 g tween-20 (Sigma). The solution was heated on a water bath at 30°C for 1 h. The pH of the solution was 7.5. This solution was used as the reference sample to which no ammonia or ammonium was added. To this solution was added 16 ul 5% NH_3 (Merck)/ml chaotropic solution to leave pH at 8.5 as the ammonia or ammonium chaotropic solution described.

The chaotropic wash I solution. To 120g Guanidine hydrochloride (Sigma) was added water to a total of 160 ml (7.5M).

The ethanol based wash II solution. To 10 ml 4M NaCl (Sigma) was added 100 ul 96% EtOH. To 800µl of this solution was added 100 ul water.

The DNA binding procedure. 50, 100 and 150 ul of whole blood (WBC 7.7) were added to 720 ul of the chaotropic lysis and binding solution. After 1 min, magnetic silica beads were added (ca 15 mg) and the solution was allowed to incubate for 10 min whereafter the magnetic beads were collected on a magnet. The beads were resuspended in washing solution I and again collected on a magnet. This

step was repeated once. The beads were resuspended and washed in washing solution II and collected on a magnet. This step was repeated once. Finally, 100 ul water was added to he beads and they were resuspended at ambient temperature for ca 2 min. The beads were collected on a magnet and the supernatant was transferred to a new tube. The yield of isolated DNA was measured on a Spectrophotometer (Perkin Elmer, Lambda EZ 201).

The results are shown in Figure 1, in which DNA yield (y-axis) is plotted in arbitrary units against µl of 5% ammonia in the chaotropic lysis and binding solution. The lysis volume is fixed at 760µl and the solid phase is fixed at 15mg.

CLAIM:

1. A process for isolating nucleic acid from a nucleic acid-containing sample, which comprises:
(a) providing a chaotrope;
(b) providing a nucleic acid binding solid phase capable of binding nucleic acid in the presence of the chaotrope;
(c) providing a source of NH_4^+ or NH_3;
(d) contacting the sample with the nucleic acid binding solid phase in the presence of a liquid phase comprising the chaotrope and the NH_4^+ or NH_3; and
(e) optionally separating the solid phase with the nucleic acid bound thereto from the liquid phase.

2. A process according to claim 1, which further comprises a step of eluting the nucleic acid from the solid phase.

3. A process according to claim 1 or claim 2, wherein the sample comprises a biological sample.

4. A process according to claim 3, wherein the biological sample comprises a cellular sample.

5. A process according to claim 3 or claim 4, which further comprises a lysis step comprising subjecting the biological sample to conditions to lyse the sample.

6. A process according to claim 5, wherein the NH_4^+ or NH_3 is present during the lysis step.

7. A process according to any preceding claim, wherein the nucleic acid comprises DNA.

8. A process according to claim 7, wherein the DNA comprises ds or ss DNA.

9. A process according to any of claims 1 to 6, wherein the nucleic acid comprises RNA.

10. A process according to claim 9, wherein the RNA comprises rRNA, mRNA or total RNA.

11. A process according to any preceding claim, wherein the chaotrope comprises a guandinium salt, urea, or an iodide, chlorate, perchlorate or (iso)thiocyanate.

12. A process according to any preceding claim, wherein the nucleic acid binding solid phase comprises a silica-based solid phase.

13. A process according to any preceding claim, wherein the solid phase is magnetic.

14. A process according to any preceding claim, wherein the source of NH_4^+ or NH_3 comprises a solution of ammonia.

15. A process according to any preceding claim, wherein the source of NH_4^+ or NH_3 and the chaotrope are provided together as a solution.

16. A kit for isolating nucleic acid from a nucleic acid-containing sample, which kit comprises:

(a) a chaotrope;

(b) a nucleic acid binding solid phase capable of binding nucleic acid in the presence of the chaotrope; and

(c) a source of NH_4^+ or NH_3.

17. A kit according to claim 16, which further comprises a solution for eluting the nucleic acid from the solid phase.

18. A kit according to claim 16 or claim 17, which further comprises a lysis solution for lysing biological samples.

19. A kit according to any of claims 16 to 18, wherein the nucleic acid binding solid phase comprises a silica-based solid phase.

20. A kit according to any of claims 16 to 19, wherein the solid phase is magnetic.

21. A kit according to any of claims 16 to 20, wherein the source of NH_4^+ or NH_3 comprises a solution of ammonia.

22. A kit according to any of claims 16 to 21, wherein the source of NH_4^+ or NH_3 and the chaotrope are provided together as a solution.

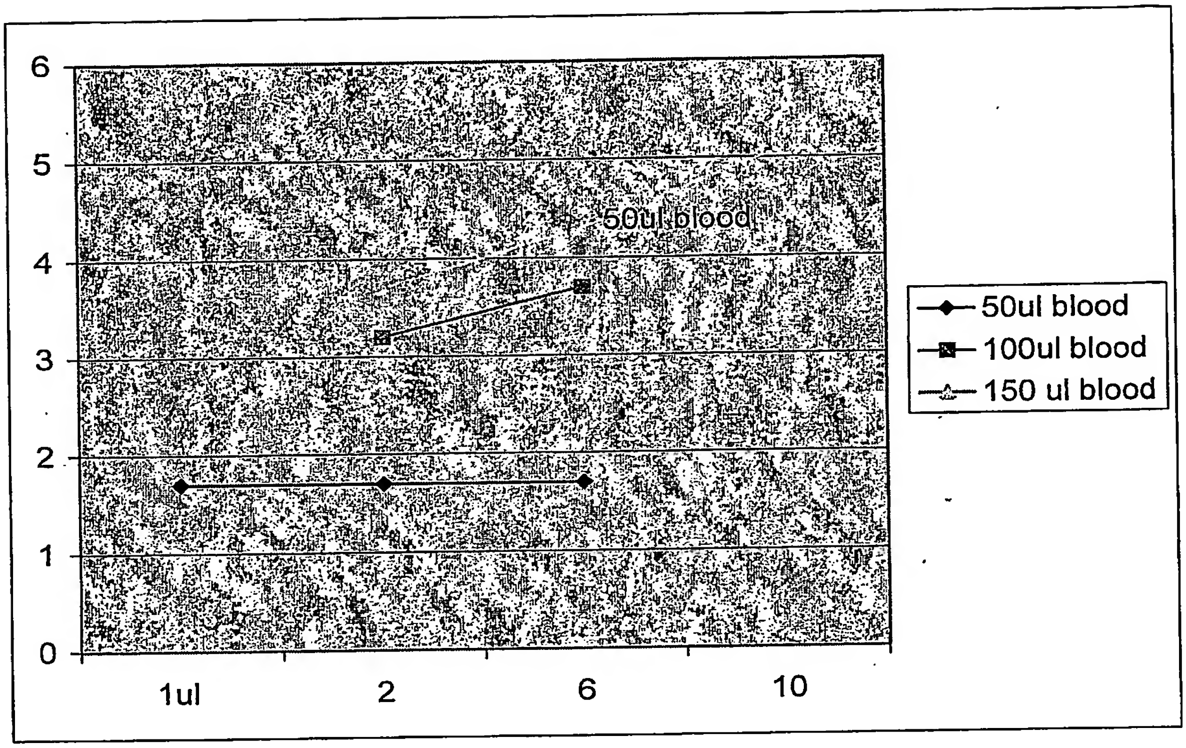
6
5
4
3
2
1
0
50ul blood
1ul
2
6
10
50ul blood
100ul blood
150 ul blood

Figure 1